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Bioluminescence Maintenance in Juvenile *Porichthys notatus*

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Abstract. Bioluminescence in the midshipman fish, *Porichthys notatus* from the Santa Barbara coastal region, was quantified from onset through the first two years of life. Maximum light emission was 2.5×10^9 photons s^{-1} upon leaving the nest and reached 2.0×10^{10} photons s^{-1} within the first year. These intensities may be sufficient for counterillumination in moon or starlight over most of the depth range of the fish. The bioluminescence of juveniles recently detached from the nest was depleted by multiple topical applications of a dilute noradrenalin solution. A luciferin-free diet also exhausted luminescence in 10–18 months. Bioluminescence was restored within 24 h after feeding depleted fish with dried specimens of the bioluminescent marine ostracod *Vargula hilgendorfii*, and light emission capacity was correlated with the amount consumed. Predation by second year fish (18 months) upon juvenile *P. notatus* (3 months) or upon live *V. tsujii* also restored luminescence. After restoration, luminescence gradually disappeared within several months. Consumption of luciferin-containing organisms by already competent fish did not increase light intensity. Juvenile *P. notatus* from the Santa Barbara coastal region require exogenous luciferin to remain luminescent.

Introduction

The midshipman fish, *Porichthys notatus*, has been the subject of many investigations since Greene (1899) first noted its bioluminescence originating from hundreds of dermal photophores, primarily confined to the ventral surface. Its natural history has been reviewed by Hubbs (1920), Arora (1948) and Ibara (1967). A nocturnal predator, it remains buried in the sand during the day and ascends at night to feed. The habitat is moderately deep waters to 400 m on the coastal shelf except during the breeding season. Then, during late spring and early summer, sexually mature adults migrate inshore to spawn in the intertidal to 80 m depths (Feder *et al.*, 1974). Males establish sheltered nesting sites and acoustically attract females. Females deposit up to 400 eggs, and multiple matings may result in the male guarding nests containing more than 1000 eggs.

P. notatus ranges from southeastern Alaskan waters to Baja California (Wilimovski, 1954) with a discontinuity along the coast of Oregon dividing the group into northern and southern divisions (Warner and Case, 1980). Although the photophores of both groups are ultrastructurally indistinguishable, the northern fish are non-luminescent (Strum, 1969). Both populations contain luciferase, but the northern population lacks luciferin (Tsuji *et al.*, 1972; Barnes *et al.*, 1973). Administration of the bioluminescent marine ostracod *Vargula hilgendorfii*, induces luminescence in Puget Sound fish (Tsuji *et al.*, 1972; Barnes *et al.*, 1973).

Fish of the southern division, south of Monterey Bay, are uniformly luminescent, while the central population, north of Monterey to Cape Mendocino, includes luminescent and non-luminescent fish (Warner and Case, 1980; Thompson and Tsuji, 1989). The analog of *V. hilgendorfii* along the western North American coast is *V. tsujii*, whose distribution coincides with the southern midshipman population (Kornicker and Baker, 1977). Its absence from northern waters led to speculation that *V. tsujii* is the luciferin source for the southern population (Warner and Case, 1980).

The inability to experimentally deplete luminescence in luminous adults despite long periods of captivity and repeated challenges with noradrenalin suggested that adult *P. notatus* have already acquired large luciferin reserves from the diet or that there is a luciferin recycling or syn-

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thesis mechanism (Barnes *et al.*, 1973). Ingestion of small numbers of luminescent ostracods rendered northern adults capable of luminescence for up to two years, with light yield greater than was considered theoretically possible for the amount of luciferin consumed, suggesting that *de novo* synthesis is triggered by exogenous luciferin or that there is a recycling mechanism (Thompson *et al.*, 1988a). A preferential mechanism for rapid uptake of luciferin from the digestive system has also been reported in *P. notatus* (Thompson *et al.*, 1988b).

The purpose of our study was to observe the effects of a luciferin-free diet on the development of bioluminescence in *P. notatus* juveniles of the southern population and to ascertain whether the fish of this population need exogenous sources of luciferin to remain luminescent. To this end, light emission was quantified from its onset in larval fish through the first two years in laboratory-reared and in locally collected individuals of *P. notatus*. The effects of multiple challenges with noradrenalin on the depletion of luminescence in the fish are described as are experiments that contributed to the evaluation of the luciferin recycling hypothesis.

Materials, Methods and Results

Collection

Breeding pairs of *P. notatus* were collected intertidally along the Pacific Coast, just north of Santa Barbara, California. They were maintained in large aquaria with sand filtered, running seawater (16–20°C). Masonry and large, inverted abalone shells provided nesting sites. Mating usually ensued during the first night in captivity and produced between 100–400 eggs. Females were removed after spawning and males were retained to maintain the nests. Additional nests with guardian males were obtained by divers. After larval detachment (35–50 days post-fertilization), juveniles were placed in aquaria with sand-covered bottoms sufficient for burrowing.

Free-living juveniles were collected with a twenty-five foot, semi-balloon otter trawl from October to May. Minimum depth of capture of first year fish ranged from 30 m in the fall to 80 m in late spring. All fish were maintained on a luciferin-free diet consisting of living *Artemia* and kelp mysids and frozen squid.

Laboratory reared and trawled fish of the same year class had similar growth rates (Fig. 1A). Photophore diameter increased directly with standard length (Fig. 1B).

Light measurement

Bioluminescence was quantified with an integrating sphere quantum counting photometer (Latz *et al.*, 1987). Small fish, less than 4 cm sl (standard length) were placed individually in a head down position in a clear plastic test

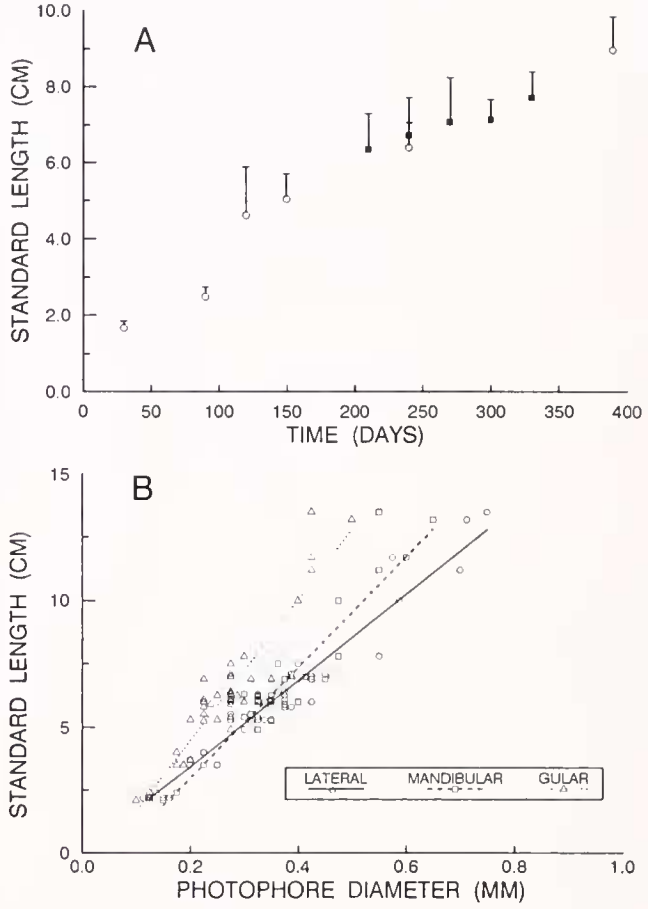

Figure 1. *Porichthys notatus* growth and photophore development. (A) Standard length (cm) *versus* time (days) post-fertilization. For each group tested, mean values are shown; error bars represent one SD of mean. Data represent laboratory raised (circles) and captured (squares) fish. For wild fish, age was determined from average time when fish left nest at capture site. Sample size ranged from 20 to 150. (B) Standard length (cm) *versus* photophore diameter (mm). The fifth anteriormost photophore of each series from the right side of the fish was measured. One specimen contributed one point for each series. Data for lateral (circles), mandibular (squares), and gular (triangles) series are presented together with calculated least-squares linear regressions of length on time according to the equations: $y = 0.31 + 17x$, $r^2 = 0.93$ (solid) for lateral; $y = -1.38 + 21.8x$, $r^2 = 0.90$ (dashed) for mandibular; $y = -1.08 + 27.8x$, $r^2 = 0.88$ (dotted) for gular.

tube (10 × 75 mm or 15 × 75 mm) inside the 0.25 m diameter integrating sphere. Approximately 6 ml of free volume remained in the test tube after the introduction of the fish. A translucent stopper, containing delivery and output intramedic tubing, sealed the tube. Twelve milliliters of freshly prepared 0.005 *M* (±)-noradrenalin (Sigma) in filtered seawater was then administered by means of a syringe attached to the input tubing externally to the sphere. The fish was completely immersed in the solution. Two minutes later, a second dose of 6 ml was administered and the overflow collected outside the

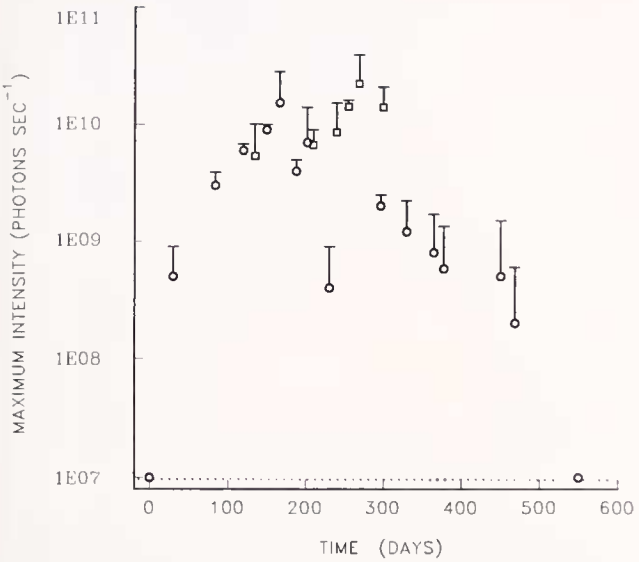

Figure 2. Bioluminescence induction and depletion. Maximum intensity (photons s^{-1}) *versus* time (days) after fertilization. Data represent laboratory raised (circles) and captured (squares) fish. Laboratory raised fish received no exogenous luciferin in the diet. Trawled fish were tested two weeks after capture. For each group tested, mean values are shown; error bars represent one SD of mean. Sample size ranged from 10 to 25. Dotted line represents noise background of integrating sphere and points on line are to be considered nonluminescent.

sphere. Larger fish were placed in a 7.0 cm diameter circular plexiglass chamber, 3.5 cm high, containing 40 ml of 0.005 *M* noradrenalin.

Bioluminescence was recorded for 5 min with a RCA 8850 photomultiplier tube operated at −1700 V and baffled so that only light reflected from the sphere internal surface was measured. This procedure is essential to accurately measure total emission from large organisms containing many non-isotrophically radiating photophores. The detector signal was processed through a discriminator calibrated at −0.315 V and the resulting frequency signal was counted with an Ortec No. 776 counter/timer and displayed on a Norland No. 5400 multichannel analyzer (MCA). Radiometric calibration of the detection system involved determination of the combined spectral responsitivity of the integrating sphere and photomultiplier tube using an Optronic Laboratory Model 310 calibration source. Light absorbance by fish within the sphere was checked against a C^{14} activated phosphore and found to be negligible. The MCA trace was stored on film or floppy disk.

Immersion in noradrenalin solution elicited bioluminescence within 20 s by transcutaneous absorption as shown by the effectiveness of topical application in air, thus avoiding mouth and gills. Luminescence was not observed prior noradrenalin application.

Maximum light emission was usually attained within 5 min and gradually declined until exhaustion approximately 40 min later. Exposure to fresh noradrenalin solutions during the trial did not appreciably raise intensity levels.

A 365 nm emitting ultraviolet (UV) lamp (UVSL 25, Ultraviolet Products Inc.) was used to qualitatively test for luciferin in photophores by observing fluorescence visually (Barnes *et al.*, 1973).

Bioluminescence depletion and induction

Luminescence was initially detected about 30 days after spawning in 1.7 cm sl larval fish still attached to the nest. Bioluminescence capacity in the laboratory population maintained on a luciferin-free diet increased for 6 months, before gradually declining (Fig. 2). Loss of luminescence capacity occurred from 10 to 18 months after spawning. Laboratory reared and trawled fish showed indistinguishable luminescence levels up to approximately 300 days.

Recently detached juvenile fish (2.5–3.5 cm sl) were exhausted of luminescence capacity by immersing them in 0.005 *M* noradrenalin for approximately 5 min every other day until no luminescent response was elicited (Fig. 3). Depleted animals remained nonluminescent unless their diet was supplemented with luciferin as described below. The remaining untreated population was tested bimonthly for luminescence with noradrenalin. For each sample of the latter group, the tests represented the first exposure to noradrenalin.

Noradrenalin-depleted fish invariably could be restored to luminescence competence by the administration of

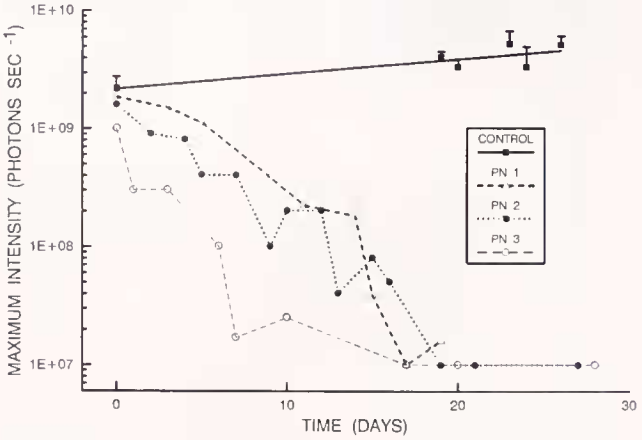

Figure 3. Noradrenalin induced depletion of bioluminescence. Maximum intensity (photons s^{-1}) *versus* time (days) after first challenge with noradrenalin. For control group tested (squares), mean values are shown; error bars represent one SD of mean. Data points represent time of first challenge with noradrenalin. Control fish received only one challenge. For the three experimental fish (circles and triangles), each point represents a challenge. Sample size of each control group was 10. Solid line represents calculated least-squares logarithmic regression of intensity on time according to equation $y = 2.3 \times 10^9 + 0.02x$, $r^2 = 0.61$.

dried *Vargula hilgendorfii* (0.7 to 4.0 mg). Depleted fish were anesthetized in tricaine methane sulfonate (MS 222, ICN-K&K Laboratories), 100 mg/l filtered seawater, and fed 6–12 whole, dried *Vargula hilgendorfii* via intramedic tubing (PE 190) slipped into the anterior gut. Controls were anesthetized but not fed. To guard against regurgitation, fish were monitored for 15 min after regaining their ability to swim. The few ostracods expelled were dried and their weight subtracted from the total.

Restored luminescence neared but never exceeded previous maximum output. Restoration was evident within 24 h, but maximal light output was usually not attained for several weeks. Total light emission was related to the amount of *Vargula* administered (Fig. 4).

Naturally depleted, second year *P. notatus* (9.0 cm sl) also had luminescence capacity restored by ingestion of dried *Vargula hilgendorfii* (5.0–9.0 mg), live *V. tsujii* (n = 3) and recently detached juvenile *P. notatus* (n = 3 to 5) (Fig. 5). For the live feedings, fish were placed in small aquaria containing either three live *V. tsujii*, or five recently detached juvenile *P. notatus* (2.5 cm sl) and monitored until prey were consumed. Restored luminescence neared but never exceeded previous maximum output. Living *Vargula* produced the most rapid onset and greatest intensities. Reinduction was not permanent, and luminescence capacity gradually disappeared with time (Fig. 5). The rise indicated after day 90 in Figure 5 for juvenile *P. notatus* feeding on other *P. notatus* is an aberration representing one fish that had been gradually losing its capacity for luminescence. The other three fish were depleted, but died before testing on day 120. Ingestion of live *V. tsujii* by already competent juveniles did not result in increased light output.

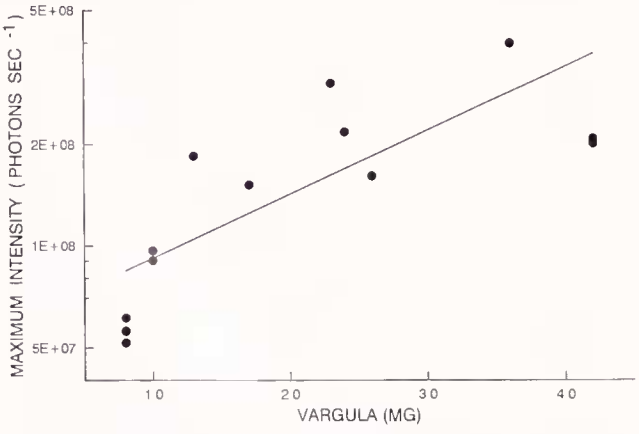

Figure 4. Maximum intensity (photons s^{-1}) *versus* weight of *Vargula* (mg) fed to depleted fish. Points represent individual fish tested 48 h after administration. Line represents calculated least-squares linear regression of light on weight of *Vargula* according to equation $y = 0.43x + 5.9 \times 10^7$, $r^2 = 0.62$.

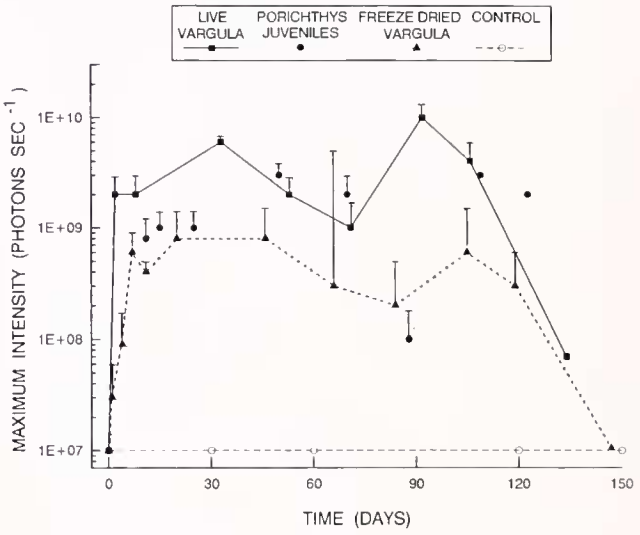

Figure 5. Maximum intensity (photons s^{-1}) *versus* time (days) after feeding with luciferin sources at day 0. Prey included live *Vargula tsujii* (squares), freeze dried *V. hilgendorfii* (triangles), and recently detached *P. notatus* (circles, dotted line). Controls are represented by circles, dashed line. Sample size ranged from 4 to 8 prior to day 90, and 1 to 4 for the rest of the experiment. Dashed line represents background of integrating sphere and points on line are to be considered nonluminescent.

Trawling also reduced or depleted luminescence. The effect was more pronounced in younger (4.2 cm sl) fish captured in November than in older fish of the same year class (6.9 cm sl) trawled the following spring (Fig. 6). Two weeks were needed for captured animals to attain maximum light levels.

Individual photophore responses

Light from individual photophores was also quantified. Thirty microliters of 0.001 *M* noradrenalin was injected under the branchiostegal photophores of a fish anesthetized with tricaine methane sulfonate MS 222 as outlined by Thompson *et al.* (1987). After a 10-min delay to insure that the fish was luminescing, 5–10 photophores of a specific anatomical series were removed surgically as a strip, and immediately placed in filtered seawater in a small, clear plexiglass chamber ($4 \times 2 \times 0.3$ cm) in the integrating sphere. Luminescence was measured for 5 min. To insure that preparation time did not bias the measurements, the test order was reversed for every other fish in the test series. All photophore series were tested within 1 h of injection.

The average light intensity of single photophores varied with location on the fish. Photophores from the gular series were significantly brighter (ANOVA: $P < 0.01$) than all other photophores except those from the branchiostegal series (Table I).

Discussion

Larval *P. notatus* from the southern population are initially luminescent. However, these experiments demon-

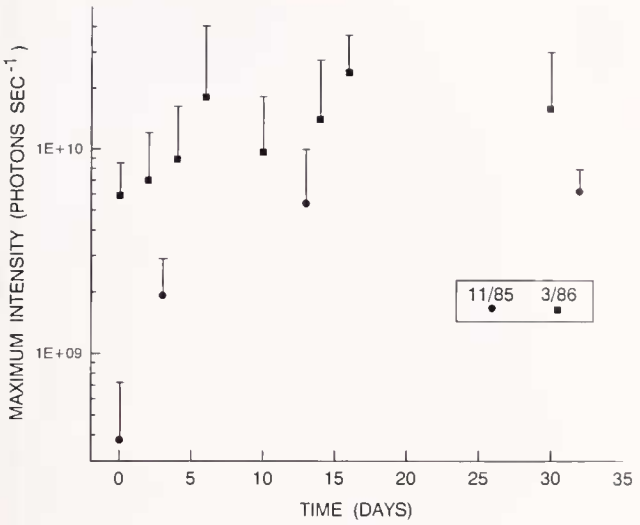

Figure 6. Bioluminescence recovery after collection by trawling. Maximum intensity (photons s^{-1}) *versus* time (days) after trawl. For each group tested, mean values are shown; error bars represent one SD of mean. All fish are from first year class. Points indicate average intensity from *Porichthys notatus* captured off Santa Barbara during 11/85 (circles) or 4/86 (squares). Average standard lengths were 4.2 cm (November) and 6.9 cm (April). The initial two points in each graph represent fish receiving first challenge with noradrenalin. Remaining points represent fish with at least one previous challenge. Sample size ranged from 12 to 25.

strate that exogenous sources of luciferin must be acquired during at least the first two years of life if luminescent capacity is to be retained. Bioluminescence was detected in smaller (1.7 cm sl) fish than has been previously reported, owing perhaps to the increased sensitivity of the integrating sphere photometer over previously used detectors, and to the fact that this photometer allows accurate quantification of luminescence for entire animals rather than only for groups of photophores as in previous studies (Tsuji *et al.*, 1972; Anctil, 1977).

The increase in bioluminescence capacity during the initial six months in laboratory animals shows the importance of maternally provided luciferin reserves. Luminescent capacity did not increase with added exogenous luciferin during this period, indicating emission capacity was not initially substrate limited. The rise in light output during juvenile life is positively correlated with increasing photophore area. Other contributing factors might be increased emission per unit area or increased efficiency of use of luciferin reserves. A comparison of light emission and standard length of laboratory and trawled fish of the same age indicated that our laboratory maintenance procedures had no detrimental effect on bioluminescence capacity or growth during the first half year.

Trawling resulted in an average 60–90% temporary reduction of light capacity with a much greater effect noted in smaller than in larger fish of the same year class. Approximately 25% of trawled fish tested negative for both fluorescence and bioluminescence within 24 h of retrieval. As even dip netting can stimulate luminescence in captive fish, it is highly likely that the stress of trawling invokes luminescence. Although trawl stress may have depressed neurological pathways controlling luminescence, the absence of fluorescence and subsequent return of luminescence indicates that the cause is luciferin depletion in the light organs and that time is required to replenish the substrate from reserves. Larger fish exhibited less reduction perhaps due to greater luciferin concentration in their larger photophores and reserves or better resistance to stress. These results strongly indicate that care should be taken in interpreting luminescence capacity of recently trawled fish, especially smaller ones, because our tests with UV and noradrenalin indicate that 25–50% of a particular trawl would be non-luminescent immediately after capture, even though all regained luminescence within a week. Our results indicate that Thompson and Tsuji (1989) may have overestimated the number of nonluminescent fish in their trawls as fish were not given time to recover.

The adaptive significance of bioluminescence in the midshipman has long been obscure. It has been speculated that the photophores attract prey by mimicking euphausiid swarms (Tsuji *et al.*, 1971). Displays have been observed during mating and in response to predators (Crane, 1965; Lane, 1967). A role in mating is unclear because females are acoustically attracted to mate and the nesting environment does not optimize the effect of bioluminescent displays. We have observed numerous successful matings in laboratory tanks and only once was luminescence briefly noted. Furthermore, the northern population reproduces without luminescence capability.

The dominantly ventral photophore position and the intensity range of light emission seems ideal for counter-

Table I

Average intensity (photons s^{-1}) of individual photophores from various anatomical series

	Individual Photophore Response	
Photophore location[1]	n	Average intensity ± 1 S.D. photons s^{-1} per photophore
Mandibular	36	$1.7 \times 10^6 \pm 1.1 \times 10^6$
Branchiostegal	32	$4.3 \times 10^7 \pm 1.6 \times 10^7$
Gular	29	$7.6 \times 10^7 \pm 4.5 \times 10^7$
Ventral	36	$2.7 \times 10^6 \pm 2.6 \times 10^6$
Pleural	33	$4.0 \times 10^6 \pm 2.9 \times 10^6$
Scapular	23	$4.4 \times 10^5 \pm 8.1 \times 10^4$

[1] According to Greene (1899).

Light was quantified for five minutes. Five to ten photophores were tested in each fish and n represents total number of photophores tested. Photophores were taken from four adult fish.

illumination. Although the *P. notatus* emission spectrum (Tsuji *et al.*, 1975) does not exactly match nocturnal astronomic light (Munz and MacFarland, 1977), this may be unimportant because many of the California nearshore fishes that feed primarily at night lack the visual pigments necessary to detect hue differences (Hobson *et al.*, 1981). Additionally, the variability in water quality of nearshore waters can alter the apparent emission spectrum. Luminescence intensity is sufficient to counterilluminate moon or starlight throughout most of the normal depth range. Upon leaving the nest, fish emit at 2.0×10^4 photons s^{-1} cm^{-2}, which is sufficient to counterilluminate starlight at approximately 30 m depth and full moonlight at 70 m depth in Class 1A oceanic water (Jerlov, 1976) on cloudless nights. The fish probably could match intensities at somewhat shallower depths because the nests are found in water usually less clear than class IA and the effects of kelp canopies or clouds are considerable.

Although counterillumination in fish has not been demonstrated in captivity, its presumed importance may explain the early onset of luminescence in larval fish that become competent to luminesce weeks before detachment. Given the inverted position of the larvae, low reflectivity of the substrate and presence of a guardian male, it is doubtful that luminescence has much functional significance on the nest. However, it may be vital to counterilluminate immediately after detachment.

This study shows, for the first time, depletion of luminescence in previously competent fish. Other investigators had been unable to deplete naturally luminous adult fish or ostracod-induced northern fish despite long periods of captivity or multiple challenges with noradrenalin (Tsuji *et al.*, 1972; Barnes *et al.*, 1973; Thompson *et al.*, 1987). In our study, luminescence was depleted by frequent challenges with topically applied noradrenalin or by maintenance on a luciferin-free diet. Noradrenalin treatment depleted luminescence within three weeks in recently detached fish, thus leading to the conclusions that frequent stimulation expends maternally acquired reserves and that a regeneration process is not present. In juvenile southern fish, lack of exogenous luciferin also depleted luciferin reserves within 10–18 months. The depletion may have been the result of spontaneous luminescence or metabolic elimination of luciferin. Loss of luminescent capacity by whatever means was not detrimental to the photophores as luminescence could be restored by luciferin administration.

The three luciferin sources used in our experiments all induced luminescence between 20 and 48 h, more than twice as quickly as reported in previous studies (Tsuji *et al.*, 1972; Barnes *et al.*, 1973). This is attributed to the smaller body size of the fish, which presumably allowed quicker substrate transport to the photophores, and improved instrumentation.

Although it has long been speculated that *V. tsujii* is the source of *P. notatus* luminescence, this is the first study in which fish were found to actively prey on *V. tsujii*, albeit in laboratory tanks. Cannibalism was also observed for the first time in *P. notatus*. First year fish are easy prey of older fish, and small conspecifics have been noted in the stomach of the closely related species, *P. myriaster* (Allen, 1982). Previously it had been thought that young fish must accumulate adequate life-time luciferin reserves before reaching maturity (Warner and Case, 1980), because it seemed unlikely that larger fish would consume small ostracods and would, at any rate, occupy deeper water outside the range of *Vargula*. Clearly, such is not the case and the overlapping ranges of first and second year fish off Santa Barbara make it possible for younger fish to be an additional exogenous source of luciferin for older conspecifics, thus establishing a link which would allow the larger fish effective indirect access to *Vargula* luciferin.

It was calculated using the methods of Thompson *et al.*, (1987) that the fish should theoretically yield approximately 2×10^{15} photons s^{-1} for the amount of dried *Vargula* ingested. Although light emission was only quantified for 5 min, none of the induced fish emitted light for over 40 min and many displays lasted less than 20 min towards the end of the study. Even using the extremely liberal calculation of multiplying the maximum intensity attained during the run by 40 min to derive photon yield, and then summing the trials for each fish, the brightest fish only produced 2×10^{13} photons s^{-1} (range 2×10^{12} to 2×10^{13} photons s^{-1}), a value two orders of magnitude below the theoretical yield. Thompson *et al.* (1988) report that the fish retain approximately 1% of the luciferin ingested. Even after estimating the luciferin retention rate at two orders of magnitude less than actually ingested, the majority of the induced fish fall short of attaining theoretical values.

These experiments do not support either a luciferin synthesis or recycling mechanism for juveniles of the southern population. Luminescence could be depleted by continual challenges with noradrenalin or maintenance on a luciferin-free diet. Although luminescence could be restored with exogenous sources of luciferin, the effect was not prolonged and emission values fell short of theoretical values.

These results contrast with the reports of Thompson *et al.* (1987; 1988a,b) who found that the persistence of *V. hilgendorfii* [^{14}C]luciferin in the photophores and long lasting luminescence after feeding small amounts of luciferin suggested an active recycling mechanism in Puget Sound adults (Thompson *et al.*, 1988b). Furthermore, they reported *Vargula* feeding to previously non-luminescent fish, yielded more light than was theoretically possible for amount of luciferin ingested.

A major difference between our investigations and those of Thompson *et al.* (1987; 1988a,b) was our use of southern juveniles instead of northern adults. These populations may have evolved different luciferin pathways in response to availability of exogenous luciferin in the diet of adults. The southern habitat includes two luciferin sources, *V. tsujii* and young *P. notatus*. Perhaps owing to this, they have lost or have never evolved an alternative mechanism, or possibly it does not function until their later, deep water stage when they are out of range of exogenous sources. If *Vargula* disappeared gradually from the northern range, the northern fish might have evolved mechanisms to maximize the effects of increasingly rare encounters with luciferin sources. Another possibility is that the pathways require minimal amounts of luciferin to function. Once levels drop below a critical concentration, enzymes needed for synthesis or recycling are no longer produced.

A second difference in these investigations is that the use of the integrating sphere photometer allowed us to quantify more accurately the total light emission per fish and avoid the assumptions concerning total light emitted, average intensity, and differential photophore emission made in previous reports (Thompson *et al.*, 1987). For example, Thompson *et al.* (1987) multiplied the directly measured intensity of 127 photophores mostly from the branchiostegal and gular series (Greene, 1899) by 4.3 to determine light emission from the whole fish, with the underlying assumption that all photophores emit equal intensities. However, we find that the branchiostegal and gular series contain the most intensely emitting photophores of the entire fish (Table I). We calculated emission based on photophore average intensity by dividing the fish into three areas: ventral photophores inside the light capture geometry of the Thompson *et al.* (1987) photomultiplier (branchiostegal and gular series; n = 127), remaining ventral and lateral photophores (mandibular, ventral, and lateral series; n = 397) and head and dorsal photophores (scapular series; n = 150). We determined total light output by taking the average intensity of the photophores contained within representative series (listed above for each area) and multiplying the average photophore intensity by the approximate number of photophores contained within each area. Our values indicate that quantification of light intensity for the whole animal by extrapolating from only the branchiostegal and gular photophores overestimates light emission by at least a factor of 3.5.

Another factor not considered by Thompson *et al.*, (1987) in arguing for luciferin recycling is that, in the course of losing luminescence capability, fish initially lose luminescence capacity in posterior photophores. Fish low in luminescence capacity were noted by dark adapted observers to have many posterior photophores unresponsive to noradrenalin application (Mensinger and Case, unpub.). Whether this is due to smaller reserves in posterior photophores or a transport mechanism that gives the anterior sites higher priority remains unknown. A mechanism favoring supply of luciferin to anterior ventral photophores during luciferin limitation would have adaptive value in preserving counterillumination capability for the larger and therefore more conspicuous, anterior regions. If the latter is true, however, the localized anterior noradrenalin injection sites used in Thompson *et al.* (1987) may have disproportionately depleted luciferin reserves from the anterior series, thus resulting in the transport of luciferin from the more distal sites. Whatever the mechanism, extrapolating intensities from anterior photophores would, therefore, result in erroneously higher emission estimates and thereby induce error into calculations of luciferin use.

We found no evidence of a mechanism for long-term maintenance of luminescence capacity in southern fish. Eventual loss of luminescence in animals without previous noradrenalin exposure rules out the possible deleterious effects of repeated noradrenalin challenges in the fish used in our investigation. The loss of luminescence capacity after induction with luciferin showed that there was no synthesis mechanism dependent on priming with nonmaternal sources of luciferin. The loss of maternally or naturally acquired reserves through spontaneous luminescence, diffusion, or autoxidation may have decreased luciferin below recyclable levels. However, the relatively short reinduction periods (3–6 months) and low photon yields cast doubt on the presence of a recycling mechanism in second year southern fish.

We conclude that fish of the southern population must continually acquire exogenous sources of luciferin, at least during their early life history, to remain luminescent.

Acknowledgments

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